

Susceptibility testing and reporting of new antibiotics with a focus on tedizolid: an international working group report

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Inappropriate use and overuse of antibiotics are among the most important factors in resistance development, and effective antibiotic stewardship measures are needed to optimize outcomes. Selection of appropriate antimicrobials relies on accurate and timely antimicrobial susceptibility testing. However, the availability of clinical breakpoints and *in vitro* susceptibility testing often lags behind regulatory approval by several years for new antimicrobials. A Working Group of clinical/medical microbiologists from Brazil, Canada, Mexico, Saudi Arabia, Russia and the UK recently examined issues surrounding antimicrobial susceptibility testing for novel antibiotics. While commercially available tests are being developed, potential surrogate antibiotics may be used as marker of susceptibility. Using tedizolid as an example of a new antibiotic, this special report makes recommendations to optimize routine susceptibility reporting.

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Widespread use and misuse of antibiotics have been associated with the selection and spread of antibiotic-resistant strains in both humans and animals. Antimicrobial resistance now poses a significant threat to public health across the world, with alarming increases in the number of infections due to multidrug-resistant pathogens [1]. Consequently, availability of new antimicrobials is now a critical unmet need. In an effort to promote the development of new, more active antibiotics, the WHO recently published its first list of antibiotic-resistant 'priority pathogens' divided into three categories – critical, high and medium. It is hoped that the publication of this list will help guide research toward pathogens that present the greatest risks to public health [2].

While research on antibiotics to combat multidrug-resistant Gram-negative pathogens (e.g., carbapenem-resistant Enterobacteriaceae) is recognized as being a critical need, high priority is also given to Gram-positive bacteria, which remain a major cause of nosocomial infections [2,3]. Methicillin-resistant *Staphylococcus aureus* (MRSA), in particular, is a prevalent threat in many parts of the world, and is often associated with significant morbidity and mortality, particularly in the elderly [4,5]. While vancomycin has been the mainstay of treatment for the management of MRSA infections [6], therapeutic failures have been reported for isolates possessing minimum inhibitory concentrations (MICs) >1.5 µg/ml [7–9]. In addition, a loading dose of vancomycin is recommended and therapeutic drug monitoring required to optimize outcomes, so complicating clinical use [10], particularly in low-resource settings. The high activity of the novel oxazolidinone antibiotic tedizolid against Gram-positive bacteria, including MRSA and vancomycin-resistant enterococci, presents a new treatment option for these challenging pathogens, providing more potent activity than linezolid *in vitro* [11–14]. Although the *optrA* gene in some strains of Enterococci may confer elevated MICs to both tedizolid and linezolid [15–17], this mechanism has not yet been identified in MRSA.

Noninferiority of tedizolid phosphate (given for 6 days) versus linezolid (10 days) has been demonstrated in two Phase III studies in patients with acute bacterial skin and skin structure infections [18,19].

Inappropriate use and overuse of antibiotics are among the most important factors in the development of resistance, and effective antibiotic stewardship measures are needed to optimize the use of antimicrobials [20]. This includes use of restrictive reporting, and/or encouraging diverse prescribing to avoid overuse of valuable antibiotics and help control the increase in antimicrobial resistance. Clinicians' ability to select appropriate antimicrobials relies on accurate and timely antimicrobial susceptibility testing (AST), a process designed to predict clinical efficacy [20]. However, performing effective AST is challenging, particularly for newly approved antibiotics, since availability of clinical breakpoints and inclusion on commercial panels and/or access to other *in vitro* susceptibility materials often lag behind regulatory approval by several years [1]. Obtaining sufficient clinical data to enable clinical breakpoints for new agents to be established is also problematic. A Working Group of clinical/medical microbiologists from Brazil, Canada, Mexico, Saudi Arabia, Russia and the UK was convened to examine issues surrounding AST for novel antibiotics. This article summarizes their recommendations for optimal routine reporting of susceptibility to new antibiotics, using the novel oxazolidinone antibiotic tedizolid as an exemplar.

Overview of testing methodology

In recent years, few new Gram-positive directed antibiotics have become available, with only dalbavancin hydrochloride, oritavancin and tedizolid phosphate being approved over the past 5 years [21–26]. In order to select the optimal therapy for their patients, clinicians must be aware of changes in resistance patterns and the effectiveness of the antibiotics used to treat particular organisms over time. For this reason, a number of global antimicrobial surveillance programs, including STAR (Surveillance of Tedizolid Activity and Resistance; tedizolid), SOAR (Survey of Antibiotic Resistance; amoxicillin/clavulanic acid, cefuroxime, cefaclor, azithromycin and ofloxacin), ZAAPS (Zyvox[®] Annual Appraisal of Potency and Spectrum; linezolid) and TEST (Tigecycline Evaluation Surveillance Trial; tigecycline), have been initiated [27–31].

Susceptibility testing can be either automated (e.g., Vitek2, Microscan and Phoenix), or manual (e.g., disk diffusion [as recommended by the European Committee on Antimicrobial Susceptibility Testing – EUCAST – or Clinical and Laboratory Standards Institute – CLSI], broth microdilution or gradient testing [e.g., E-test[®]]). Each system has advantages and limitations, with some providing quality control ranges [1,32–35]. Working Group members report that the majority of laboratories in their countries use automated testing systems routinely for susceptibility testing, most of which provide MICs. Manual testing is frequently used to confirm automated AST results and/or MICs; E-test[®] and disk diffusion are the preferred methods, with the latter being the more favored choice in cost-conscious environments. Of note, in routine susceptibility testing, vancomycin MICs may not reflect accurately the actual MICs and their confirmation by E-test[®] has recently been recommended to minimize the risk of treatment failure [36]. Determination of MICs is important for certain strains not identified by an automated system or disk diffusion (e.g., vancomycin-intermediate *S. aureus* [VISA]). Failure with empiric vancomycin treatment in a Saudi 69-year-old male patient, for example, prompted physicians to obtain more accurate susceptibility data on the pathogen isolated from blood. This led to the discovery of the first heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA), a threatening signal to the effectiveness of empiric vancomycin treatment in hospitalized patients with severe infections [37].

Effective performance of AST by clinical microbiology laboratories is essential in order to determine susceptibility to the chosen empirical antimicrobial agents, and to detect the emergence of resistance [38]. Use of susceptibility breakpoints (e.g., those provided by the CLSI and EUCAST) is important, both for consistent reporting of antimicrobial susceptibility and for consistency and comparability of international surveillance schemes [39]. EUCAST and CLSI also advocate use of epidemiological cut-off values as one of several tools in the process of establishing clinical breakpoints; determination of such cut-off values may promote more susceptibility testing and allow MIC values to be put into context [40]. While most laboratories follow either CLSI or EUCAST breakpoints for susceptibility testing, some may use both if a pathogen is not included in the guidelines they normally follow. When there is no available information on appropriate clinical breakpoints for certain bacteria, physicians may rely on their best judgment with regard to MIC findings and experience in eradicating those pathogens, and/or on consultation with clinical/medical microbiologists or infectious disease specialists.

Most laboratories in the Working Group members' countries report an isolate's susceptibility to be 'susceptible', 'resistant' or 'intermediate' according to either CLSI or EUCAST criteria. The 'intermediate' category has been used in a number of various ways, for example as a buffer to prevent very major and major errors, to be used alongside

the 'resistant' category to indicate results that are 'nonsusceptible' and to indicate pathogen or drug combinations where an increased drug exposure may be necessary for optimal results [40]. However, antibiotic decision-making is usually based on 'susceptible' or 'resistant' categories, since treatment choices for 'intermediate' susceptibility are not clear-cut. 'Intermediate' should be interpreted as 'nonsusceptible' and physicians should thus choose an alternative antibiotic to which the pathogen is susceptible. Nevertheless, reporting susceptibility as 'intermediate' can be useful in some cases, for example if two antibiotics are used synergistically (e.g., meropenem and colistin) or to optimize the antimicrobial dosage regimen [41].

Selection of antibiotics for routine susceptibility testing depends primarily on the type of AST method, particularly if an automated panel is used. All antibiotics included on the panel are routinely tested, although not all of those tested may be reported; reports are usually issued only for first-line antibiotics. Until the cause of an infection is known, initial therapy is generally empiric and guided by clinical presentation [20]. Clinical/medical microbiologists and infectious disease specialists have an important role in advising physicians on the most appropriate antibiotics to use, although decision-making can also involve infectious disease specialists and clinical pharmacologists. The number and selection of antimicrobials tested is dependent on the organism isolated, infection site, the institution's formulary, physician requests and the automated panel or other testing methodology used [38]. Notably, the choice of antibiotics to be tested via automated methods can be limited, including for new antibiotics that have yet to be adopted by automated systems. While inclusion of an antibiotic on the hospital formulary is a key factor in selection for susceptibility testing, availability on the automated panel, supply of the manual testing equipment (disks or gradient strips) and requests from physicians are also important.

The inclusion of new antibiotics on commercial, automated test panels is often delayed for a considerable time after approval [38,42]. However, agents from the same class with similar activities can be used as surrogate markers (i.e., class representatives) to predict susceptibility of clinical isolates to new agents and/or to those not included in routine testing. Clinical laboratories have used surrogate testing successfully for decades (see Table 1). Reliability of a surrogate marker is typically analyzed by testing the categorical agreement between the susceptibility results for the two agents, defining errors as very major (i.e., false-susceptibility), major (i.e., false-resistance) or minor (i.e., the result for one agent was intermediate while the other agent was susceptible, nonsusceptible or resistant) [43]. However, it should be noted that the chance of a very major error is extremely low when the occurrence of resistant isolates is rare or absent. Furthermore, in rare cases, susceptibility to a surrogate antibiotic might not reveal resistance to the agent in question, for example a recently recognized, uncommon variant of *fexA* conferring resistance to florfenicol, but not to chloramphenicol [44]. Such reports highlight the need to review the utility of surrogate susceptibility testing as new data emerge.

Testing of new antibiotics: a focus on tedizolid

An alternative approach to susceptibility testing is needed for new antibiotics as their inclusion on commercial AST panels is often delayed for several years after approval, which complicates testing and reporting [1]. Despite inclusion on hospital formulary, the use of new antibiotics may be restricted for some months, which limits the opportunity for physicians to gain clinical experience. This may be difficult to explain to physicians if the antibiotic is already included in clinical practice guidelines. Consequently, clinical/medical microbiologists have a responsibility to provide clinically relevant information to physicians and explain why it is or is not appropriate to use an antibiotic. Additional barriers to susceptibility testing of new antibiotics include: problems with the availability and regular supply of materials required for manual testing; not being included on the hospital formulary; lack of breakpoints for certain pathogens; and the increased workload and costs associated with supplementary testing. Working Group members report that novel antibiotics will most likely be routinely tested once they become available on an automated panel(s). Until then, susceptibility testing for new agents is performed manually, but often undertaken only upon physician request. In the short term, surrogate agents (that are included on automated panels) can be used to predict pathogen susceptibility to new antibiotics [1].

Several methods are currently available to test tedizolid susceptibility, including the use of linezolid as a surrogate antibiotic. Linezolid susceptibility is considered a highly reliable surrogate for tedizolid susceptibility; a high categorical agreement has been reported between the susceptibility of tedizolid and linezolid; and the very major error rates were low ($\leq 0.2\%$) for all organisms tested [42]. Based on these findings, EUCAST recommends that isolates susceptible to linezolid can be reported as susceptible to tedizolid [47]. For isolates intermediate/resistant to linezolid, an MIC test must be performed to confirm susceptibility or resistance to tedizolid. Most laboratories in Working Group members' countries would add to the AST report a 'tedizolid susceptible' result based on a

Table 1. Antibiotics used as surrogate agents in clinical practice.

Surrogate agent	Pathogen	Antibiotic(s) for which susceptibility is reported	Ref.
Ampicillin	Enterococci	Amoxicillin-clavulanic acid, ampicillin-sulbactam, piperacillin, piperacillin-tazobactam	[45]
Cefepime	<i>H. influenzae</i>	Ceftaroline	[46]
Cefoxitin	Staphylococci	Oxacillin	[45]
		Cephalosporins [†]	[47]
Ceftazidime	<i>H. influenzae</i>	Ceftaroline	[46]
Ceftriaxone	<i>S. pneumoniae</i>	Ceftaroline	[46]
	<i>H. influenzae</i>	Ceftaroline	[46]
Cephalothin	Enterobacteriaceae	Cefapirin, cephadrine, cefaclor, cefadroxil, cefpodoxime, cephalexin and loracarbef	[45,48]
Ertapenem	Enterobacteriaceae <i>Haemophilus</i> spp. <i>S. pneumoniae</i>	Doripenem	[49]
Erythromycin	Streptococci	Azithromycin, clarithromycin and dirithromycin	[45]
Imipenem	<i>S. aureus</i>	Ceftaroline	[46]
	<i>P. aeruginosa</i>	Doripenem	[50]
Levofloxacin	<i>S. pneumoniae</i>	Fluoroquinolones	[51]
Linezolid	Staphylococci Enterococci Streptococci <i>Streptococcus anginosus</i> group	Tedizolid	[42]
	Staphylococci Streptococci		[47]
Meropenem	<i>S. aureus</i>	Ceftaroline	[46]
	<i>P. aeruginosa</i>	Doripenem	[50]
Nalidixic acid	<i>H. influenzae</i> <i>M. catarrhalis</i>	Ciprofloxacin, levofloxacin, moxifloxacin and ofloxacin	[47]
Norfloxacin	Staphylococci Streptococci	Ciprofloxacin, levofloxacin, moxifloxacin and ofloxacin	[47]
Oxacillin	<i>S. aureus</i>	Cefazolin, ceftriaxone, ceftaroline and nafcillin	[52]
Penicillin	Enterococci	Ampicillin, amoxicillin, amoxicillin-clavulanic acid, ampicillin-sulbactam, piperacillin, piperacillin-tazobactam	[45]
Teicoplanin	Staphylococci Streptococci	Dalbavancin	[53]
Tetracycline	<i>Vibrio cholerae</i>	Doxycycline [‡]	[45]
	Staphylococci Streptococci <i>H. influenzae</i> <i>M. catarrhalis</i> <i>N. gonorrhoeae</i>	Doxycycline and minocycline	[47]
Vancomycin	Staphylococci Streptococci	Dalbavancin	[53,54]
	Staphylococci Streptococci	Dalbavancin and oritavancin	[47]
	Staphylococci Streptococci Enterococci	Oritavancin	[55]

^{††}Not cefixime, ceftazidime, ceftibuten and ceftolozane-tazobactam.

Disk diffusion should not be used for doxycycline due to poor correlation with minimum inhibitory concentration results.

surrogate 'linezolid susceptible' actual testing result, but would not report a 'tedizolid resistance' result without knowledge of the results of actual tedizolid susceptibility testing. AST results are typically discussed with the treating physician before reporting so as not to delay clinical decision-making. The CLSI recommends that tedizolid and linezolid are included in the Group B optional primary test and reported selectively [45]. Additional methods available for tedizolid susceptibility testing include MIC test strips (Liofilchem[®], Liofilchem s.r.l., Roseto, Italy) and a broth microdilution device (Sensititre[™] Thermo Scientific[™], Thermo Fisher Scientific Inc., Cleveland, OH, USA) developed primarily for research purposes [56]. Use of 20 µg disk diffusion has been approved by the CLSI for measurement of tedizolid susceptibility as a quality control measure [45]. To obtain susceptibility results in microbiology laboratories, currently a more practicable 2 µg disk diffusion method is under development, in addition to automated susceptibility testing panels. It should be noted that methodological testing issues have been observed with tedizolid and linezolid which suggest that 80% inhibition MIC end point criterion should be employed for testing both agents [57].

Tedizolid has demonstrated more potent activity *in vitro* than linezolid against Gram-positive bacteria, including MRSA and vancomycin-resistant enterococci, as well as noninferiority in patients with skin and skin structure infections [11–14,18–19]. Of note, tedizolid retains *in vitro* activity against *S. aureus* and other Gram-positive bacterial strains that harbor the *cf*-gene encoded methylase enzyme [58,59]. This methylase enzyme confers resistance against five structurally different antibiotic classes (e.g., clindamycin in the lincosamide class, chloramphenicol in the phenicol class, and linezolid, but not tedizolid, in the oxazolidinone class) [58]. The encoded enzyme methylates the A2053 nucleotide in the peptidyl-transferase center of the 23S ribosomal RNA, which is a very highly conserved site, and this methylation prevents binding of antibiotics to peptidyl-transferase center [60]. Importantly, chromosomal mutations in Domain V of rRNA or ribosomal L3 protein identified to date were demonstrated to confer resistance to both linezolid and tedizolid, stressing the need to test tedizolid susceptibility [59,61]. The Working Group stresses the importance of providing tedizolid susceptibility results in order to guide clinicians in selecting the most effective agent for their patients, and to provide an alternative option in cases of resistance development. They recommend the adoption of EUCAST guidelines on use of linezolid as a surrogate to predict tedizolid susceptibility [47], in order to enable routine inclusion of tedizolid in AST reports by microbiology laboratories. The Working Group also advocates having tedizolid MIC test strips in place in laboratories so that it is possible to perform tedizolid susceptibility testing, particularly when nonsusceptibility to linezolid is detected. Furthermore, they highlight the importance of a surveillance program for monitoring resistance and appropriate antibiotic use of all new antibiotics.

Conclusion & recommendations

The Working Group has highlighted the difficulty in acquiring sufficient clinical data to enable clinical breakpoints for new antibiotics to be established and the time taken (possibly up to 5 years) for such agents to be included in automated testing panels, which are often used for routine susceptibility testing. Policy makers should aim to minimize delays in the adoption of new breakpoints for antibiotics against emerging pathogens, particularly when containment of spread is vital; delays should be reduced to less than 1.5 years whenever possible [62]. However, to guide clinicians on appropriate treatment, it is critical that the susceptibility of new antibiotics is reported. Using tedizolid as an example, if it is not possible to determine susceptibility, data may be extrapolated by using linezolid as a surrogate, allowing subsequent appropriate use of tedizolid. In situations identified by a clinical/medical microbiologist (e.g., linezolid nonsusceptible strains of Gram-positive bacteria) an appropriate manual test for tedizolid will be necessary (Figure 1). Susceptibility testing and routine reporting of selected new antibiotics can be desirable to ensure that clinicians make the appropriate choices for the management of infection, and that development of resistance is closely monitored as new agents become available.

Future perspective

At present, there is a considerable time lag between approval of new antibiotics, availability of clinical breakpoints and inclusion on commercial AST panels. It is hoped that closer coordination in the coming years between those involved in drug development and AST panels and regulatory authorities will shorten this delay, enabling routine testing of new agents at the time of approval. In the meantime, to ensure appropriate antibiotic choice, it is essential that laboratories use the available tools to enable them to report susceptibility of new antibiotics.

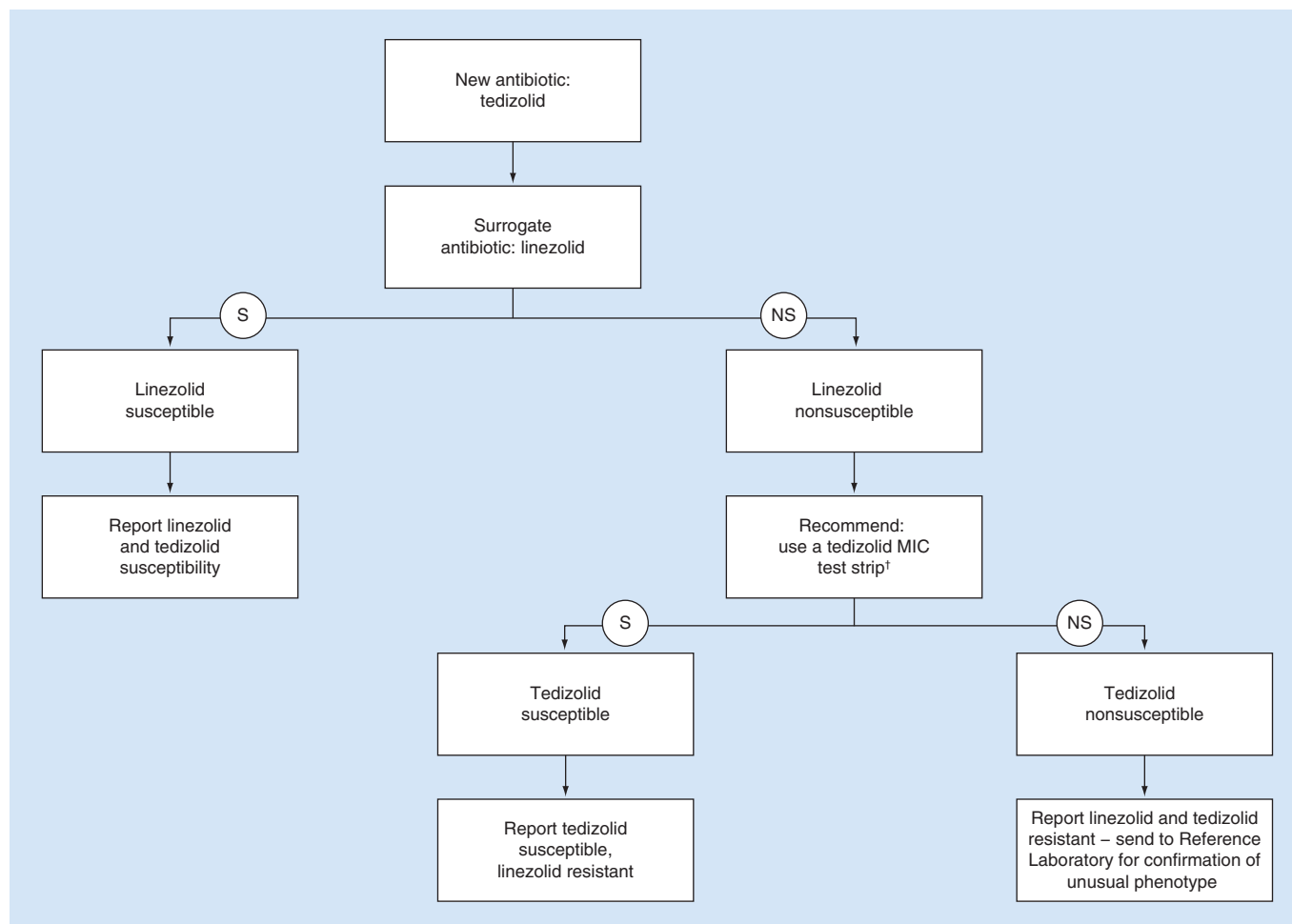


Figure 1. Recommended approach for routine reporting of susceptibility of new antibiotics, for example, tedizolid.

†Use of broth microdilution is also applicable.

As recommended by published evidence/according to susceptibility testing guidance [42,47].

NS: Nonsusceptible; S: Susceptible.

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Executive summary

Background

- A Working Group of clinical/medical microbiologists from Brazil, Canada, Mexico, Saudi Arabia, Russia and the UK has made recommendations for optimal routine testing and reporting of susceptibility to new antibiotics, using the novel oxazolidinone antibiotic tedizolid as an example.

Overview of testing methodology

- Most laboratories use automated testing systems (e.g., Vitek2, Microscan and Phoenix) routinely for susceptibility testing, with manual testing (e.g., EUCAST or CLSI disk diffusion, broth microdilution or gradient testing [e.g., E-test®]) used to confirm the results and minimum inhibitory concentrations (MICs).
- The inclusion of new antibiotics on commercial, automated test panels and/or the availability of susceptibility testing materials, are often delayed for a considerable time after approval; however, agents from the same class with similar activities can be used as surrogate markers to predict susceptibility of clinical isolates to new agents and/or to those not included in routine testing.

Testing of new antibiotics: a focus on tedizolid

- Several methods are currently available to test tedizolid susceptibility: use of linezolid as a surrogate antibiotic, MIC test strips (Liofilchem®, Liofilchem s.r.l., Roseto, Italy) and a broth microdilution device (Sensititre™ Thermo Scientific™, Thermo Fisher Scientific Inc., Cleveland, OH, USA).
- Adoption of EUCAST guidelines on use of linezolid as a surrogate to predict tedizolid susceptibility is recommended in order to enable routine inclusion of tedizolid in antimicrobial susceptibility testing reports; having tedizolid MIC test strips in place in laboratories is also advocated so that tedizolid susceptibility testing can be performed when nonsusceptibility to linezolid is detected.

Conclusion & recommendations

- It can take up to 5 years for new antibiotics to be included in automated testing panels yet it is critical that the susceptibility of these agents is reported.
- If it is not possible to determine susceptibility, data may be extrapolated by using a surrogate, for example, linezolid susceptibility as a reliable surrogate for tedizolid.
- Susceptibility testing and routine reporting of selected new antibiotics is desirable to ensure that clinicians make the appropriate choices for the management of infection.

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